

REMARKS

Reconsideration and withdrawal of the rejections set forth in the Office Action dated October 5, 2007 are respectfully requested.

I. Amendments

Claims 2, 3, 5, 7 and 28 are cancelled without prejudice.

Claim 1 is amended to incorporate the limitations of cancelled claims 2, 3 and 5.

Claims 1 and 27 are amended to add the element reciting "wherein the step of holding the cell under the pressure different from the atmospheric pressure is a step of subjecting the cell to depressurization, wherein the depressurization step is performed under a pressure reduced by about 0.096 MPa from the atmospheric pressure". Support for this amendment can be found in the specification as filed in WO 2004/007736, for example, on page 33, lines 5-18; page 38, lines 20-23; and page 60, lines 5-7.

Claims 1, 27, and 29 are amended to recite "conditions to induce electroporation". Support for this amendment can be found in the specification as filed in WO 2004/007736, for example, on page 25, lines 12-23.

The amendment of claim 6 that is directed to the range "10 V/cm to 200 V/cm" finds support, for example, on page 24, lines 30-31 of the specification as filed in WO 2004/007736.

Claim 27 is amended to incorporate the limitations of now-cancelled claim 28. Claims 29 and claim 47 are amended to depend from claim 27. Support for the element of amended claim 27 that recites "a method for introducing nucleic acids into the cells of a plant" is found, for example, on page 5, lines 6-11.

II. Claim Objections

The objection for being in improper form for multiple dependent claim serving as a basis for another multiple dependent claim has been addressed by the cancellation of claim 28 and by the amendment to claims 29 and 47.

III. Rejection under 35 U.S.C. §112, second paragraph

Claims 1-48 and 71 were rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

The rejection of claims 1, 27, 29, and their dependent claims is addressed by amending claims 1, 27, and 29 to recite "conditions to induce electroporation".

The rejection of claim 6 for having the term "high" is addressed by the foregoing amendment to claim 6.

The rejection of claim 27 is addressed by the amendment to a method for introducing nucleic acids into a plant.

IV. Rejection under 35 U.S.C. §102

Claims 1-4 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Dodgson et al. (WO 00/63407, hereafter "Dodgson") Claim 1 is now amended to include the element of depressurization performed under a pressure reduced by about 0.096 MPa from the atmospheric pressure. Dodgson does not disclose this depressurization element. Since Dodgson does not disclose each and every element of claim 1 and its dependent claims, Applicants respectfully submit that this rejection be withdrawn.

Claims 1-4 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Schmukler et al. (U.S. Patent 5,173,158, hereafter "Schmukler") Claim 1 is now amended to include the element of depressurization performed under a pressure reduced by about 0.096 MPa from the atmospheric pressure. Schmukler does not disclose this depressurization element. Since Schmukler does not disclose each and every element of claim 1 and its dependent claims, Applicants respectfully submit that this rejection be withdrawn.

Claims 1-5 and 7 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Rickwood (WO 01/05994). As discussed above, Claim 1 is now amended to include the element of depressurization performed under a pressure reduced by about 0.096

MPa from the atmospheric pressure. Since the specification discloses that one atmospheric pressure is 0.1 MPa, a pressure of 0.096 MPa from atmospheric pressure is 0.004 MPa, which corresponds to 4×10^3 Pa. This pressure is not within the range of 1×10^4 Pa to atmospheric pressure disclosed by Rickwood. Since Rickwood does not disclose each and every element of the claimed subject matter, Applicants respectfully submit that this rejection be withdrawn.

IV. Rejection under 35 U.S.C. §103

Claims 1-48 and 71 were rejected under 35 U.S.C. §103 as allegedly obvious over Dodgson in view of Gutierrez-Armenta et al. (U.S. Patent Publication 2002/0046416, hereafter "Gutierrez-Armenta").

Claims 1-48 and 71 were rejected under 35 U.S.C. §103 as allegedly obvious over Schmukler in view of Gutierrez-Armenta.

Claims 1-5, 7-48, and 71 were rejected under 35 U.S.C. §103 as allegedly obvious over Rickwood in view of Gutierrez-Armenta.

This rejection is respectfully traversed in view of the foregoing amendments and the following remarks.

A. The Present Claims

The present claims relate to a method for transferring a nucleic acid into a plant cell, comprising the steps of a) holding the cell under a pressure different from an atmospheric pressure; and b) placing the cell and the nucleic acid under conditions to induce electroporation wherein the step of holding the cell under the pressure different from the atmospheric pressure is a step of subjecting the cell to depressurization, wherein the depressurization step is performed under a pressure reduced by about 0.096 MPa from the atmospheric pressure.

The presently-claimed subject matter has the following advantages. The method makes it possible to transform plant cells without requiring a culturing step and allows for the possibility of rapidly obtaining a large amount of nucleic acid-transferred matter, particularly a whole transformant plant.

B. Analysis

A determination of obviousness is informed by an analysis of several factors: (1) the scope and content of the prior art; (2) the differences between the claimed invention and the prior art; (3) the level of ordinary skill in the art; and (4) any relevant secondary considerations. (*Graham v. John Deere Co.*, 383 U.S. 1, 17, 86 S. Ct. 684, 15 L.Ed.2d 545 (1966)).

1. Scope and content of the cited art and knowledge generally available

DODGSON discloses a method of electroporation in which depressurization is used to position cells on mesh in a container, as shown in Figure 3 and described on page 4. The size of the channel disclosed is 50 μm , preferably 30 μm . Dodgson neither shows nor suggests enhanced transfection efficiency by depressurizing cells in order to produce a large amount of nucleic acid-transferred matter. Furthermore, the channel size is large enough to retain a cultured animal cell, but not large enough to retain a plant cell.

SCHMUKLER discloses a method of electroporation but does not show or suggest that the disclosed method is effective to introduce nucleotides into cells having a cell wall. Furthermore, Schmukler discloses a method to retain cells on film having pores that are smaller than the cells such that single cells in culture can be retained. The disclosed method is not applicable to plant seeds since they are much too large to be retained on the film.

RICKWOOD discloses a method of electroporation but does not show or suggest that the disclosed method is effective to introduce nucleotides into cells having a cell wall. The method disclosed in Rickwood involves use of air bubbles to penetrate a cell membrane, particularly that of an animal cell, to introduce genetic material. Rickwood does not show or suggest that the disclosed method can be successfully applied to plant cells to produce a large amount of nucleic acid-transferred matter, particularly a whole transformant plant.

GUTIERREZ-ARMENTA discloses the use of retinoblastoma protein to control growth of plant cells and/or plant viruses. Several methods of administering nucleotides to cells are disclosed, including electroporation of plant seed cells with DNA. The reference does not show or suggest the step of subjecting the cell to depressurization,

wherein the depressurization step is performed under a pressure reduced by about 0.096 MPa from the atmospheric pressure. Furthermore, the reference presents no data indicating successful introduction of nucleotides into plant cells using electroporation.

2. Reasonable Expectation of Success

Consistent with application of the Graham factors, a patent is not obvious if there was no reasonable expectation of success.

Rejection over Dodgson in view of Gutierrez-Armenta

In the Office Action, the combination of Dodgson and Gutierrez-Armenta is alleged to demonstrate that there is a reasonable expectation of success in transferring nucleic acids into cells of plants of the types recited in the instant claims 1-48 and 71. Claim 1 is amended to be directed to the transformation of plant cells and to further incorporate the element of depressurization performed under a pressure reduced by about 0.096 MPa from the atmospheric pressure.

Dodgson nowhere shows or suggests that depressurization of plant cells during transfection is effective to introduce nucleic acids into said plant cells. There is no teaching or suggestion regarding the use of depressurization.

Gutierrez-Armenta only discloses the possibility of electroporation of plant cells with DNA in paragraph 15, without any discussion as to whether such a technique is effective or not. The teaching of Gutierrez-Armenta contradicts the teaching of the instant patent application in which "[c]onventional electroporation methods are applicable only to cells inherently having no cell wall and cells whose cell wall has been artificially removed[.]" (page 3, lines 7-9). Gutierrez-Armenta does not present any data or give any showing that electroporation methods can be used to successfully introduce nucleic acids into plant cells.

Applicants provide a manner of transfecting plant cells using electroporation by holding the cell under the pressure different from the atmospheric pressure, wherein the pressure is reduced by about 0.096 MPa from the atmospheric pressure. In the attached Declaration, the data presented in paragraph 8 indicates that the method of transfecting according to the claimed subject matter is effective to transform rice with the pWI-GUS plasmid. The results obtained are not predicted by the disclosures of Dodgson and Gutierrez-Armenta.

Since the disclosures of Dodgson and Gutierrez-Armenta combined do not indicate a reasonable expectation of success, Applicants respectfully submit that above rejection is improper.

Rejection over Schmukler in view of Gutierrez-Armenta

In the Office Action, the combination of Schmukler and Gutierrez-Armenta is alleged to demonstrate that there is a reasonable expectation of success in transferring nucleic acids into cells of plants of the types recited in the instant claims 1-48 and 71.

Schmukler does not show or suggest depressurization of plant cells to a pressure 0.096 MPa below atmospheric pressure. The disclosure of column 3, lines 20-26 relates to pressure being used to trap cells in pores rather than to enhance electroporation of plant cells. There is no disclosure that modulating pressure is effective to introduce nucleic acids into plant cells.

The disclosure of Gutierrez-Armenta is discussed above, along with Applicants' results. The results obtained, as described in the attached Declaration, are not predicted by the disclosures of Schmukler and Gutierrez-Armenta.

Rejection over Rickwood in view of Gutierrez-Armenta

In the Office Action, the combination of Rickwood and Gutierrez-Armenta is alleged to demonstrate that there is a reasonable expectation of success in transferring nucleic acids into cells of plants of the types recited in the instant claims 1-48 and 71.

Rickwood does not show or suggest depressurization of plant cells to a pressure 0.096 MPa below atmospheric pressure. The specification of Rickwood does not describe the penetration of the cell wall of a plant seed using pressurization and depressurization. Furthermore, there is no disclosure that modulating pressure is effective to introduce nucleic acids into plant cells.

The disclosure of Gutierrez-Armenta is discussed above, along with Applicants' results. The results obtained, as described in the attached Declaration, are not predicted by the disclosures of Rickwood and Gutierrez-Armenta.

In summary, because the skilled artisan would not have reasonably expected to successfully achieve the presently claimed subject matter, the standard for obviousness has not been met. Applicants submit that in the absence of hindsight and without the

Applicants' own disclosure, the selection of each of these features from the various cited documents would not be obvious to one of skill in the art.

Due to lack of reasonable expectation of success, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §103.

IV. Conclusion

In view of the foregoing, the claims pending in the application comply with the requirements of 35 U.S.C. § 112 and patentably define over the applied art. A Notice of Allowance is, therefore, respectfully requested. If the Examiner has any questions or believes a telephone conference would expedite prosecution of this application, the Examiner is encouraged to call the undersigned at (650) 838-4300.

Respectfully submitted,
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